STUDIES ON THE RELATION OF TETANUS BACILLI IN THE DIGESTIVE TRACT TO TETANUS ANTITOXIN IN THE BLOOD.

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In a previous paper (1) we have reported the isolation of *Bacillus tetani* from 34 per cent of the stools of 78 individuals in Peking, and we have discussed the relation of this high carrier incidence to the number of infections with tetanus bacilli. It is difficult to draw any conclusions regarding this relation as it is impossible to get accurate statistics, but we have been impressed by the statement of a number of foreign physicians practising in North China who agree in saying that they have seen very few cases of tetanus. In order to explain why infections with this organism are relatively uncommon in a region where a third of the population are eliminating tetanus bacilli in their stools and where human feces are so generally distributed, we asked ourselves the question: Do tetanus bacilli in the digestive tract cause the production of antibodies, and in particular antitoxins, which protect the host against infections with these organisms? In this paper we wish to report on the presence of tetanus antitoxin in the serum of individuals who carry tetanus bacilli in their digestive tracts.

When one considers the importance of this subject the records of the examination of the blood of man and animals for tetanus antitoxin are remarkably scarce. We have been able to find only the following, though there may be incidental observations buried in the extensive literature on tetanus in general. Hamburger (2) says that he has never found a trace of tetanus antitoxin in the blood of normal animals or man. Since he does not give any experimental evidence to support his statement, we do not know how many specimens he examined or what methods he used, but it is worthy of note that this is the only statement regarding the examination of human blood that we have found. Although Buxton and Glenny (3) failed to find tetanus antitoxin in the blood of 500 horses, we know from the observations of Sanchez-Toledo and Veillon (4), Choukévitch (5), Lukas (6), and

Noble (7) that these animals not infrequently carry tetanus bacilli in their digestive tracts. However, Römer (8) has examined the blood of normal cattle for antitoxin and found it present in 13 of the 39 animals studied. Of 17 cattle under 2 years of age only 1 showed antitoxin and this was a calf aged 1½ months whose mother had a high antitoxin content in her blood. Of the 22 animals over 2 years old, 12 showed antitoxin and, in some cases, large amounts of it. Römer examined the stools of only 1 of the cows having antitoxin and found tetanus bacilli present, but since others had shown that a large percentage of cattle carried tetanus bacilli in their digestive tracts and since he found antitoxin only in the blood of the older cattle, he assumes that this antitoxin is due to the presence of the bacilli in the digestive tract. We feel that his explanation is probably correct but that it would be more convincing had he shown that the cattle having antitoxin in their blood carried tetanus bacilli, while those having no antitoxin did not carry these organisms.

Method Used in Testing Sera for Antitoxin.

In all of our tests for antitoxin we have used portions of a single lot of dried tetanus toxin, prepared by the method of Brieger and Fraenkel (9), that was kept in a vacuum over phosphorus pentoxide. The M.L.D. of this toxin has remained remarkably constant at about 0.01 mg. throughout our work. For test animals we have used field mice as they are larger and more easily obtained than white mice. Per gram of body weight the former are more susceptible than the latter, as shown in the results of one of our experiments given in Table I, and it will also be seen that the M.L.D. for a 12 gm. white mouse is about the same as that for a 25 gm. field mouse.

For each day's test 0.1 gm. of toxin was carefully weighed and suspended in sterile 50 per cent glycerol, and dilutions from this suspension were mixed with equal quantities of the serum to be tested which was diluted 1:5. After incubation at 37°C. for 30 minutes, each mixture was injected into two field mice, each mouse receiving 1 cc. subcutaneously near the root of the tail. A complete protocol of the examination of one serum is given in Table II. Since the field mice varied considerably in weight we made it a practise to use on a single day those that weighed within 5 gm. of one another. Control mice of the same weight were always inoculated. As a rule, three specimens of sera from individuals who carried tetanus bacilli and three from individuals who did not were tested on the same day. After 4 days the final record on the animals was made, and those surviving were not used subsequently.

TABLE I.

Test to Compare the Resistance, to Tetanus Toxin, of Field and White Mice. March 15, 1922.

Field mice.			White mice.		
Weight of mouse.	Toxin injected.	Result.	Weight of mouse.	Toxin injected.	Result.
gm.	*mg.*		*gm.*	*mg.*	
21.5	0.005	Dead in 4 days.	12	0.005	Dead in 3½ days.
21.5	0.005	" " 3½ "	14.5	0.005	Generalized tetanus; survived.
20	0.0075	" " 3½ "	13	0.0075	Dead in 3½ days.
21	0.0075	" " 66 hrs.	12	0.0075	" " 48 hrs.
20.5	0.01	" " 40 "	12	0.01	" " 66 "
22.5	0.01	" " 48 "	11.5	0.01	" " 48 "

TABLE II.

***Protocol** of the Examination, for Antitoxin, of the Serum of a Normal Woman Who Showed Tetanus-Like Bacilli in Her Feces.*

Weight of mouse.	Amount of toxin injected.	Amount of serum injected.	Results.
gm.	*mg.*	*cc.*	
21	0.02	0.1	No effect.
21	0.02	0.1	" "
23	0.05	0.1	" "
21	0.05	0.1	" "
22	0.1	0.1	" "
21	0.1	0.1	" "
22	0.25	0.1	" "
21	0.25	0.1	" "
22	0.5	0.1	Generalized tetanus; dead in 66 hrs.
22	0.5		
20	0.01	Control.	Slight spasms 40 hrs.; dead in 3½ days.
23	0.01	"	" " 40 " " " 4 "
23	0.02	"	Spasms 40 hrs.; dead in 66 hrs.
20	0.02	"	" 40 " " " 66 "
25	0.03	"	" 40 " " " 48 "
24	0.03	"	" 40 " " " 48 "

Results Obtained from the Examination of 56 Individuals.

Our first tests were made on the sera of individuals from whose stools we had either isolated tetanus bacilli or had failed to find these

TABLE III.

Summary of the First Series of Examinations Showing the Relation of Tetanus Spores in the Feces to Antitoxin in the Blood.

Patient No.	*B. tetani* in stool.	Effect of injection of 0.1 cc. of serum mixed with tetanus toxin as indicated.						
		Date of test.	Toxin injected.		Mouse A.		Mouse B.	
					Weight.	Result.	Weight.	Result.
		1922	*mg.*	M.L.D.	*gm.*		*gm.*	
340	Isolated.	Jan. 10	0.05	5	28	No effect.	21	No effect.
			0.1	10	21	t; recovered.	21	t; recovered.
			0.25	25	20	ttt + $3\frac{1}{2}$ days.	25	ttt + $3\frac{1}{2}$ days.
374	"	" 10	0.1	10	20	No effect.	18	t; recovered.
			0.25	25	26	ttt + $3\frac{1}{2}$ days.	20	ttt + $3\frac{1}{2}$ days.
451	Absent.	" 10	0.02	2	29	ttt + 4 "	38	ttt + 66 hrs.
Controls.			0.005	$\frac{1}{2}$	24	t; recovered.	36	t; recovered.
			0.01	1	27	ttt + 5 days.	26	ttt + $3\frac{1}{2}$ days.
			0.02	2	25	ttt + 40 hrs.	26	ttt + 72 hrs.
317	Isolated.	Jan. 11	0.1	10	25	No effect.	3	No effect.
			0.25	25	21	ttt + 66 hrs.	29	t; recovered.
932	"	" 11	0.25	25	25	No effect.	21	No effect.
			0.5	50	26	t; recovered.	28	t; recovered.
172	"	" 11	0.05	5	35	No effect.	28	No effect.
			0.1	10	25	t; recovered.	22	t; recovered.
			0.25	25	26	ttt + 66 hrs.	32	ttt + 66 hrs.

138	Absent.	Jan. 11	0.02	2	31	ttt + 66 hrs.	22	ttt + 3½ days.
288	"	" 11	0.02	2	34	ttt + 3½ days.	25	ttt + 3½ "
439	"	" 11	0.02	2	42	ttt + 3½ "	22	ttt + 66 hrs.
Controls.		" 11	0.005	½	26	t; recovered.	25	t; recovered.
			0.01	1	27	tt; recovered.	26	ttt + 4 days.
			0.02	2	31	ttt + 42 hrs.	24	ttt + 66 hrs.
152	Isolated.	Jan. 17	0.1	10	25	No effect.	25	+ 66 hrs. Not in tetanic condition.
			0.25	25	26	ttt + 40 hrs.	27	ttt 40 hrs.
391	"	" 17	0.25	25	26	No effect.	25	No effect.
			0.5	50	25	ttt + 40 hrs.	25	ttt + 40 hrs.
228	Absent.	" 17	0.02	2	25	tt; recovered.	27	ttt + 4½ days.
			0.05	5	28	ttt + 66 hrs.	27	ttt + 40 hrs.
393	"	" 17	0.02	2	28	ttt + 3 days.	29	ttt + 4 days.
394	"	" 17	0.02	2	28	ttt + 4 "	27	ttt + 3½ "
Controls.		" 17	0.01	1	28	ttt + 4 "	27	ttt + 3 "

t indicates slight spasm of one leg; tt, moderate tetanus; ttt, generalized tetanus.

organisms. We have summarized these tests in Table III, giving only the largest amount of toxin that was neutralized by each and the smallest amount that was not neutralized. Although all of the specimens of sera were tested against the amounts of toxin given in Table II, the complete protocols are not given because of their length and the fact that they would add little to the value of the report. Our results show that in not a single instance did 0.1 cc. of serum from seven individuals whose stools were free from tetanus bacilli neutralize 2 M.L.D., whereas the same amount of serum from the seven individuals whose stools contained tetanus bacilli neutralized 10 M.L.D. of toxin, and in one case (No. 932) 0.1 cc. practically neutralized 50 M.L.D. of the toxin.

TABLE IV.

Summary of the Tests Made on the Sera of 42 Individuals, Showing the Relation of Tetanus-Like Bacilli in the Feces to Antitoxin in the Blood.

Result of stool examination.	No. of sera with the maximum amount of tetanus toxin neutralized by 0.1 cc.					
	2 M.L.D. not neutralized.	2 M.L.D.	5 M.L.D.	10 M.L.D.	25 M.L.D.	50 M.L.D.
Tetanus-like bacilli present.	0	1	1	4	10	3
" " absent.	21	0	0	2	0	0

In our previous work we had shown that we could almost invariably isolate tetanus bacilli whenever the stained film of the sediment from a fermentation tube inoculated with the heated suspension of the feces showed typical tetanus-like organisms. Since this isolation of tetanus bacilli from mixtures containing great numbers of other anaerobes is a difficult and time-consuming process, in our next series of tests we determined the presence or absence of tetanus-like bacilli in the feces and did not attempt to isolate them. The sera of nineteen individuals whose stools contained tetanus-like organisms and the sera of twenty-three whose stools were negative were tested for antitoxin as in the preceding series. The results of these tests are summarized in Table IV, and they show that in all of the cases where tetanus-like organisms were found in the feces, there was a definite neutralization of the toxin by the serum. On the other hand, the toxin was neutralized by the sera from only two of the twenty-three individuals whose stools were apparently free from tetanus bacilli.

If we combine these two series, we find that all of the sera from the twenty-six individuals whose feces showed tetanus-like bacilli contained antitoxin, while of the thirty whose stools were free from these organisms, only two did so; 0.1 cc. of the sera of these two neutralized 10 M.L.D., while the same amount of the remaining twenty-eight failed to neutralize 2 M.L.D. of toxin.

Percentage of Individuals Having Antitoxin in Their Serum.

As the relation of antitoxin in the serum to tetanus bacilli in the intestinal tract is so constant we have a means of checking our previous work on the percentage of individuals who act as carriers of this organism. We secured from the Wassermann laboratory forty specimens of serum, most of them from normal individuals, such as employees of the institution and soldiers from one of the legations. From each specimen two mice were inoculated subcutaneously, each mouse receiving a mixture that contained 0.1 cc. of serum and 10 M.L.D. of toxin. Of the forty specimens tested, fifteen, or 37.5 per cent, neutralized the 10 M.L.D. of toxin, a figure that agrees very closely with the results of our stool examination which showed the presence of tetanus bacilli in 34 per cent of the individuals examined.

Estimated Antitoxin Content of Serum of Carriers of Tetanus Bacilli.

We have been unable to compare our toxin with accurately standardized antitoxin, but we have tested it against two lots of antitoxin put up for therapeutic purposes. One lot from the Bureau of Science, Manila, where the United States Army unit is used, required 0.1 unit to neutralize 100 M.L.D of our toxin. The other lot from the Kitasato Institute for Infectious Diseases, where the German unit is used, required 0.002 unit to neutralize 100 M.L.D. of the same toxin. These figures agree well with MacConkey's (10) statement that the German unit equals 40 United States Army units, but we feel that they are only approximate as the strength of antitoxin may vary greatly from the figure on the container. We can say, however, that individuals who carry tetanus bacilli in their digestive tracts have an appreciable amount of antitoxin in their blood since 0.1 cc. of most of our sera neutralized 10 and, in many cases, 25 M.L.D. of toxin. We hope that someone who has access to accurately standardized toxin will study the blood of carriers of tetanus bacilli and determine more accurately the amount of antitoxin present.

DISCUSSION.

Our question as to whether the presence of tetanus bacilli in the digestive tract leads to the production of antibodies by the host is thus answered in the affirmative, and we can understand why in a region where a third of the population carry these bacilli, infections are relatively uncommon. We can also understand why we find so few cases of tetanus following dysentery, typhoid, and operations on the intestine.

A carrier of tetanus bacilli is potentially a disseminator of these organisms, but he is not so great a menace to his fellow men as the horse, because his feces are as a rule disposed of more promptly. On the other hand, it is to his advantage to become a carrier for he thereby acquires an immunity, which, while it may not be absolute, may be of great service to him should tetanus bacilli enter his body through a wound.

If we could be sure that ingested spores would, in every case, grow in the intestines, we should have an easy method of immunizing a large body of men to tetanus bacilli. Tulloch's findings (11) indicate that many of the men who were in France during the recent war became carriers of tetanus bacilli, and it is probable that many lives were thereby saved. Would it have been wise to have made all of these men carriers so that they could have produced their own immune bodies? From what we see in China, we might think so, but we must not forget that such a procedure would result in a wide distribution of tetanus bacilli, the evil effects of which might counterbalance the good effects of the immunization. The immunity resulting from the ingestion of spores is probably only relative, and if bacilli in great numbers were introduced into wounds from badly contaminated soil it is probable that the body would be overwhelmed.

It is probable, therefore, that a better procedure, in case of another great war, would be to immunize men by the injection of toxin-antitoxin mixtures as used by Buxton and Glenny (3) for protecting horses against tetanus.

Our attempt to study the production o fantitoxin following the establishment of tetanus bacilli in the human digestive tract has as yet yielded no satisfactory results. For this experiment one of us swallowed a large number of mature spores. A period of marked constipation followed, which may or may not have been due to the presence of tetanus bacilli in the intestines. Before the spores were ingested no tetanus bacilli were present in the feces, while for the first 10 days after they were ingested, they were present in great numbers. At this time it became necessary to take a drastic cathartic on account of the constipation, and afterwards no more spores were found in the feces. We were more successful in a monkey and later we will report the studies made on the blood of this animal after the establishment of tetanus bacilli in its digestive tract.

It seems possible that the explanation of the varying susceptibility of guinea pigs to tetanus toxin as noted by Taber (12) is due to the fact that these animals may carry tetanus bacilli in their digestive tracts (7). Whether these carriers develop antitoxin is a question we have not investigated, but it seems probable that they behave just as men do in this respect.

Whether the antitoxin found in the blood of carriers of tetanus bacilli is due to absorbed toxin or to the occasional entrance of the organisms into the body is a question we cannot answer. If, however, the latter were the case, we should expect cases of idiopathic tetanus to be relatively common; yet so far we have been unable to get a single record of idiopathic tetanus in this region.

From the clinical standpoint, a quick method of testing for antitoxin in the blood is desirable. The agglutination test cannot be used for we find that sera free from antitoxin will often agglutinate the type of tetanus bacillus that predominates here in as high dilutions as does serum containing antitoxin. Neutralization tests are not practical as a common laboratory procedure for the toxin must be freshly made up for each test, its strength must be determined, and the results of the test are not known for some days. However, should it be desirable to test a large body of men to determine their immunity, the injection of 0.1 cc. of serum and 10 M.L.D. of toxin into mice is practical and is a comparatively simple procedure.

The finding of agglutinins of tetanus bacilli in sera free from antitoxin may help us in answering a question that has puzzled us for some time. As we have stated above, tetanus infections are relatively infrequent here, yet our results show that only approximately a third of the population have tetanus antitoxin in their blood. The remainder of the population certainly have had numerous opportunities to ingest the bacillus and the bacilli have either been unable to grow because of antagonistic organisms, or they have grown and been cast off. The finding of agglutinins in the blood of a certain percentage of individuals whose serum contains no antitoxin and whose stools are free from the bacillus points to the fact that the latter explanation is the correct one. If this is the case, we must assume that the agglutinins have persisted longer than the antitoxins, and then the question arises as to whether these people really are immune. We should expect their bodies to be trained in antitoxin production, and in case of an infection with tetanus bacilli to respond more quickly than the body of an individual who had never had tetanus bacilli in his digestive tract or produced antitoxin. Glenny and his associates (13, 14) have shown that this quick response occurs in animals that have previously been immunized with diphtheria toxin. At the time of the second injection of toxin their blood may be free from antitoxin, but they produce it much more rapidly than a normal animal. The assumption is that they are actively immune. We therefore feel that the relatively low incidence of tetanus in North China is due, in part, to the fact that the majority of the population have a potential immunity because they carry or have carried tetanus bacilli in their digestive tracts. Other factors may be the intense sunlight, which may sterilize the soil, and the few industrial accidents.

CONCLUSIONS.

1. The sera of twenty-six individuals who carried tetanus bacilli in their digestive tracts all contained appreciable amounts of antitoxin.

2. The sera of thirty individuals in whose stools no tetanus-like organisms were found were, with two exceptions, free from tetanus antitoxin.

3. Although we have been unable to measure accurately the antitoxin content of these human carriers of tetanus bacilli, 0.1 cc. of serum neutralizes 10 or more M.L.D. of toxin and it is evident that they have acquired an active immunity due to the bacilli in the intestinal tract.

4. These results definitely prove that tetanus bacilli grow in the intestinal tract of man.

5. Many of the individuals who have no tetanus bacilli in their intestinal tracts and whose serum is free from antitoxin show agglutinins to tetanus bacilli. It is probable that they have been carriers of the bacilli in the past and that the agglutinins have persisted longer than the antitoxins. It seems likely, therefore, that these individuals are potentially immune to tetanus.

6. If tetanus bacilli can be established in the digestive tract of man we have a means of immunization which might be useful in armies or in regions where tetanus infections are common, though we do not recommend this method of immunization at present.

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